



## EFFECT OF GLYPROLINES ON THE LEVEL OF APOPTOTIC AND NEUROTROPHIC FACTORS UNDER CONDITIONS OF “SOCIAL” STRESS

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**The aim** of the article was to study the effect of glyproline neuropeptide compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu, on the level of apoptotic factors (caspase-3, caspase-8, the tumor necrosis factor) and neurotrophic factors (the nerve growth factor and the brain neurotrophic factor) in the blood serum of white rats under the experimental modeling of “social” stress.

**Materials and methods.** The experimental studies were carried out on 90 nonlinear white male rats aged 6 months. By the type of behavior, in the process of “social” stress modeling, all the rats were divided into “aggressors” and “victims”. In the study, the following experimental groups (n=10) were formed: control individuals; groups of the rats exposed to stress for 20 days; groups of the animals treated intraperitoneally at the dose of 100 µg/kg/day, starting from the 1st day of the stress factor exposure, with a course of 20 days of glyproline compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu. The effect of the compounds on the level of apoptotic and neurotrophic factors was assessed by determining the level of caspase-3, caspase-8, the tumor necrosis factor, the nerve growth factor and the brain neurotrophic factor of white rat blood serum by enzyme immunoassay.

**Results.** According to the results of the study, it was found out that under the conditions of “social” stress, there was an increase in the apoptotic processes accompanied by an increase in the level of caspase-3, caspase-8, TNF-α in the blood serum of white rats, as well as a decrease in the concentration of neurotrophic factors – BDNF and NGF. The administration of glyproline compounds against the background of stress, contributed to the restoration of the studied indicators level, which is most likely due to the presence of antiapoptotic and neuroprotective effects in glyprolines due to the inhibition of the caspase-dependent cascade of apoptosis reactions, as well as the induction of the synthesis of neurotrophic factors with the antiapoptotic activity.

**Conclusion.** Thus, the administration of glyproline neuropeptide compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu under stress conditions, contributes to the restoration of the initiating and effector caspases level, as well as of neurotrophic factors. As a result of the experiment, an anti-apoptotic effect is observed due to the inhibition of the caspase-dependent cascade of reactions, as well as a stress-protective effect is observed due to the restoration of the brain neurotrophic factors level.

**Keywords:** glyprolines; neuropeptides; “social” stress; apoptosis; caspases; tumor necrosis factor; brain neurotrophic factor; nerve growth factor

**Abbreviations:** TNF-α – tumor necrosis factor; NGF – nerve growth factor; BDNF – Brain-derived Neurotrophic Factor; CNS – central nervous system; cIAP – cellular inhibitor of apoptosis proteins.

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## ВЛИЯНИЕ ГЛИПРОЛИНОВ НА УРОВЕНЬ АПОПТОТИЧЕСКИХ И НЕЙРОТРОФИЧЕСКИХ ФАКТОРОВ В УСЛОВИЯХ «СОЦИАЛЬНОГО» СТРЕССА

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**Цель.** Изучить влияние глипролиновых нейропептидных соединений Thr-Lys-Pro-Arg-Pro-Gly-Pro (Селанк), Pro-Gly-Pro и Pro-Gly-Pro-Leu на уровень апоптотических (каспаза-3, каспаза-8, фактор некроза опухоли) и нейротрофических (фактор роста нервов и нейротрофический фактор головного мозга) факторов в сыворотке крови белых крыс в условиях экспериментального моделирования «социального» стресса.

**Материалы и методы.** Экспериментальные исследования проводили на 90 нелинейных белых крысах-самцах 6-месячного возраста. В процессе моделирования «социального» стресса все крысы были разделены по типу поведения на «агрессоров» и «жертв». В исследовании формировались экспериментальные группы (n=10): контрольные особи; группы крыс, в течение 20 дней подвергавшиеся воздействию стресса; группы животных, получавших внутривенно в дозе 100 мкг/кг/сут, начиная с 1-го дня воздействия стресс-фактора, курсом 20 дней глипролиновые соединения Thr-Lys-Pro-Arg-Pro-Gly-Pro (Селанк), Pro-Gly-Pro и Pro-Gly-Pro-Leu. Влияние соединений на уровень апоптотических и нейротрофических факторов оценивали путем определения уровня каспазы-3, каспазы-8, фактора некроза опухоли, фактора роста нервов и нейротрофического фактора головного мозга сыворотки крови белых крыс методом иммуноферментного анализа.

**Результаты.** По результатам проведенного исследования было установлено, что в условиях «социального» стресса наблюдалось усиление апоптотических процессов, сопровождающихся увеличением уровня каспазы-3, каспазы-8, TNF-α в сыворотке крови белых крыс, а также снижение концентрации нейротрофических факторов: BDNF и NGF. Введение глипролиновых соединений на фоне стресса способствовало восстановлению уровня исследуемых показателей, что, вероятнее всего, связано с наличием у глипролинов антиапоптотического и нейропротекторного действия за счет ингибирования каспаза-зависимого каскада реакций апоптоза, а также индукции синтеза нейротрофических факторов, обладающих антиапоптотической активностью.

**Заключение.** Таким образом, введение глипролиновых нейропептидных соединений Thr-Lys-Pro-Arg-Pro-Gly-Pro (Селанк), Pro-Gly-Pro и Pro-Gly-Pro-Leu в условиях стрессогенного воздействия способствует восстановлению уровня иницирующей и эффекторной каспаз, а также нейротрофических факторов. По итогу проведенного эксперимента наблюдается антиапоптотический эффект за счет ингибирования каспаза-зависимого каскада реакций, а также стресс-протекторный за счет восстановления уровня нейротрофических факторов мозга.

**Ключевые слова:** глипролины; нейропептиды; «социальный» стресс; апоптоз; каспазы; фактор некроза опухоли; нейротрофический фактор мозга; фактор роста нервов

**Список сокращений:** TNF-α – фактор некроза опухоли; NGF – фактор роста нервов; BDNF – нейротрофический фактор головного мозга; ЦНС – центральная нервная система; cIAP – клеточный ингибитор белков апоптоза.

### INTRODUCTION

At present, scientific works reflecting the results of studying the pathological influence of stress factors, including “social” stress, on various body systems, are of particular interest [1, 2]. The recent studies prove the fact that a prolonged exposure to stress contributes to the formation of neurological, immune, endocrine, oxidative, metabolic and other types of disorders; that ul-

timately leads to the development of violations of the molecular and cellular mechanisms of a programmed cell death, including apoptosis of neurons. [3, 4]. To date, close attention is paid to assessing the role of apoptotic and neurotrophic factors in the implementation of the stress response.

The most informative indicators of apoptotic processes assessment are initiator and effector caspases,

which activate each other and trigger the caspase cascade [5–7]. It has been proven that when the body is exposed to stress factors, the apoptotic processes of neurons are activated due to the initiation of effector caspase-3 by caspase-8 [8–10]. The established initiation process is characteristic of the apoptosis development in lymphoid and endothelial cells, which, in turn, contributes to the development of immune dysfunction, as well as pathology of the cardiovascular, urinary and other systems [11, 12]. It has been proven that as a result of the exposure to stress factors, the development of caspase-dependent apoptosis is observed, which, to an even greater extent, is aggravated by the accumulation of free radicals. A caspase-dependent pathway, or the pathway of “death receptors” located on the cell surface, is characteristic of intact cells; the mitochondrial pathway mediated by the Bcl-2 family of proteins is characteristic of pathologically transformed cells. The pathway of “death receptors” is regulated by cytokines and is shorter than the other pathway – mediated by mitochondria, but functionally both of them are closely related to each other.

A tumor necrosis factor (TNF- $\alpha$ ) is an equally important participant in apoptotic processes. It was found out that, as a result of the exposure to stress factors, the formation of a TNF- $\alpha$  complex with Fas receptors is observed. It is followed by triggering of signaling molecules, which activate caspase-3 and 8, leading to irreversible damage to neurons [12]. It has been proven that, alongside with a pronounced pro-inflammatory activity, a tumor necrosis factor contributes to an increase in the secretion of inflammatory mediators and the induction of apoptosis, due to binding to receptors on the target cell membrane, in particular to the membrane receptor TNF-R2 [13, 14]. In this case, TRAF2 molecules are inactivated, and, in their turn, they support the process of triggering cIAP apoptosis inhibitor proteins. In addition, TNF- $\alpha$  causes the cell death by the necrosis mechanism, promoting the formation of reactive oxygen species, which cause the destruction of membranes and death of the target cell. The above mentioned makes it possible to classify this cytokine as one of the important participants in apoptosis. It has been proven that a tumor necrosis factor plays an important role in the pathogenesis of diseases such as myocardial infarction, chronic renal failure, bronchial asthma [14], and reveals its initiating effect on the development of autoimmune pathology [13]. It was found out that the tumor necrosis factor is increased in the patients with neuropsychiatric and neurodegenerative diseases, as well as a traumatic brain injury [15].

When considering the neurotrophin hypothesis of various pathological disorders development (including apoptosis), such neurotrophic factors as the nerve growth factor and the brain neurotrophic factor, which have a pronounced neurospecificity, are of great importance in the manifestation of a neuropro-

TECTIVE effect. This action is realized due to the ability of the neurotrophic factors to induce the synthesis of anti-apoptotic proteins and inhibition of pro-apoptotic ones, thereby influencing the survival and differentiation of individual neurons populations. A number of studies reflect a direct dependence of apoptosis on the balance of NGF and BDNF, which activate the receptors of tyrosine kinases and have a neuroprotective effect [16–18].

It has been established that the nerve growth factor attracts the attention of scientists as a promising kind of treatment for various neuropsychiatric diseases such as Alzheimer’s disease and depression [19]. The recent research results indicate that, alongside with a direct effect on the nervous system, NGF has a multifactorial effect on the body [20–22]. The nerve growth factor plays a key role in the regulation of regeneration processes. That is due to its influence on the mechanisms of maintaining homeostasis, inflammation, proliferation and tissue remodeling. The ability of the nerve growth factor to induce the release of immunoactive neuropeptides and neurotransmitters, as well as to influence innate and adaptive immune responses, has been proven [20, 21]. It should be notified that the level of the brain neurotrophic factor expression reflects the treatment effectiveness of hypoxic-ischemic, traumatic and toxic lesions of the central nervous system [22]. It has also been established that the level of BDNF serum has a negative correlation with the severity of anxiety disorders and even determines the development of neurodegenerative processes in some cases [23, 24].

Thus, apoptotic and neurotrophic factors play the role of active participants in the implementation of adaptive mechanisms to stress effects of various origins and determine the prospects of considering them as a target for pharmacological agents with a stress-protective activity [24].

Currently, neuropeptide compounds with a versatile pharmacological activity, including a stress-protective activity, are of particular interest [25]. A large number of highly effective and safe drugs are synthesized based on neuropeptides [26]. It should be notified that neuropeptides are capable of penetrating the blood-brain barrier and exerting a pharmacological effect at minimal concentrations [27]. Being modulators of physiological processes, peptide preparations are able to control the expression of cellular messengers and cytokines, thereby influencing the initiation of apoptotic processes in the nervous system and performing the antiapoptotic protection function [28]. To date, in a series of peptide compounds, regulatory peptides of a glyproline nature have been isolated as a separate group [29]. Its most significant representative is the registered drug Selank (Trademark No. 199370), synthesized by scientists of the Institute of Molecular Genetics of the National Research Center “Kurchatov Institute” by a merger with Pro-Gly-Pro to the C-termini

of tuftsin, originally used as an immunomodulator [30]. In practical medicine, Selank is used to improve mnemonic functions [31], providing antiasthenic, adaptogenic, antihypoxic [32] and actoprotective effects [33]. Currently, domestic scientists from leading scientific organizations are studying in detail the pharmacological action of glyprolines [27–35]. It has been established that this class of peptides is able to prevent atherosclerotic processes and reduce thrombus formation by activating the fibrinolytic and anticoagulant mechanisms [33]. The studies have shown that glyproline peptides have a hepatotropic effect [34]. The results of a number of the experimental data have demonstrated hypoglycemic and hypolipidemic effects of these compounds [27]. The Pro-Gly-Pro tripeptide itself, which has a pronounced physiological activity, is of considerable interest from the perspective of a promising therapeutic agent [30]. Numerous works have established that the uniqueness of glyproline neuropeptides lies in their pleiotropy, i. e., in the combination of psycho- [25], neuro- [35], nootropic effects [26]. The presence of the immunotropic activity of glyprolines has been proven [36]. It is confirmed by their participation in the induction of various neurotrophic factors, pro- and anti-inflammatory cytokines, and the regulation of apoptosis processes [34, 35]. The properties described above actualize the need for a detailed study of the pharmacological action of the neuropeptides of the glyproline structure.

**THE AIM** of the article was to study the effect of glyproline neuropeptide compounds Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu, on the level of apoptotic factors (caspase-3, caspase-8, the tumor necrosis factor) and neurotrophic factors (the nerve growth factor and the brain neurotrophic factor) in the blood serum of white rats under experimental modeling of “social” stress.

## MATERIALS AND METHODS

### Laboratory research

The experimental studies were carried out on 90 white male rats aged 6 months, obtained from the vivarium of the laboratory of physiology, morphology, genetics and biomedicine of Astrakhan State Medical University (Russia, Astrakhan). Keeping laboratory animals met the requirements of regulatory documents<sup>1,2,3</sup>. The experiment was carried out on the basis of the protocol of the Ethics Committee of Astrakhan State Medical University No. 8 dated November 24, 2015.

<sup>1</sup> Directive of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes (2010/63/EU). Saint Petersburg, 2012. – 50 pp. Russian.

<sup>2</sup> The International Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986). Russian.

<sup>3</sup> Order of the Ministry of Health of the Russian Federation No. 199n dated 01.04.2016. “On the approval of the Rules of laboratory practice”.

### Experimental model

The model of “social” stress was implemented by providing living arrangements of rats where there is a sensory contact and no physical contact, followed by the formation of aggressive and submissive types of behavior [35]; herewith, the animals were placed in pairs in the cages separated by a transparent partition. In order to observe inter-male confrontations, the partition was removed daily for 10 minutes, according to the results of which the groups of rats “aggressors” and rats “victims” were formed. In the experimental animals, the manifestation of aggression was expressed in the forms of upright and sideways offensive postures – “threat” or attack. In addition, submissiveness was manifested by various acts of individual behavior: immobility, sniffing, autogrooming, and upright defensive postures [37–39].

### Experimental groups

In the study, the following experimental groups (n=10) were formed: control individuals; groups of the rats exposed to stress for 20 days; groups of the animals treated intraperitoneally at the dose of 100 µg/kg/day, starting from the 1st day of the stress factor exposure, with a course of 20 days of glyproline compounds Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu.

The choice of the glyproline compounds dose was based on a preliminary study of the severity of the psychomodulatory effect by assessing behavioral reactions using psychopharmacological settings. The studies were carried out with the administration of glyproline compounds at the doses of 25, 50, 100, and 200 µg/kg/day. It was found out that glyprolines were most active at the doses of 100 and 200 µg/kg/day. In this connection, subsequently, the lowest experimental dose of 100 µg/kg/day was chosen.

### Methods

The effect of neuropeptides of the glyproline structure on the level of caspase-3, caspase-8, the tumor necrosis factor, the brain neurotrophic factor and the nerve growth factor in the blood serum of white rats was assessed by the method of enzyme-linked immunosorbent assay using an immunological analyzer “Multiscan FC” and a highly sensitive ELISA Kit for Caspase -8 (USA); ELISA Kit for Caspase-3 (USA); ELISA Kit for Tumor Necrosis Factor Alpha (TNF-α) (USA), ELISA Kit for Brain Derived Neurotrophic Factor (BDNF) (USA); ELISA Kit for Nerve Growth Factor (NGF) (USA). Before use, the serum was kept at room temperature for two hours, centrifuged for 20 minutes at 1000 rpm, and then immediately subjected to analysis. The choice of this type of biological material was made, based on the analysis of the literature data [40, 41].



### Statistical processing of results

Statistical processing of the research results was carried out using the software packages Microsoft Office Excel 2007, BIostat 2008 Professional 5.8.4.3 taking into account the Mann-Whitney criterion. The differences were considered statistically significant at  $p \leq 0.05$ .

### RESULTS AND DISCUSSION

The results reflecting the effect of glyprolines on the level of caspases-3 and 8 in the blood serum of white rats under conditions of "social" stress are presented in Table 1.

While the formation of "social" stress in the animals with an aggressive type of behavior was taking place, the level of caspase-3 increased by 1.8 times ( $p \leq 0.01$ ) in relation to the control. While the introduction of glyproline neuropeptide compounds was taking place, a decrease in the studied indicator was notified: with Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank) – by 1.6 times ( $p \leq 0.01$ ), with Pro-Gly-Pro – by 1.5 times ( $p \leq 0.01$ ) and with Pro-Gly-Pro-Leu – by 1.3 times ( $p \leq 0.01$ ) in comparison with the stress group.

In the group of the stressed animals with a submissive type of behavior, the level of caspase-3 increased by 60% ( $p \leq 0.01$ ) in relation to the control group. With the administration of Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), there was a decrease in the indicator by 50% ( $p \leq 0.01$ ), with Pro-Gly-Pro – by 29% ( $p \geq 0.05$ ) and with Pro-Gly-Pro-Leu – by 10% ( $p \geq 0.05$ ) in comparison with the "social" stress group.

The formation of "social" stress led to an increase in the level of caspase-8 by 2.6 times ( $p \leq 0.01$ ) in comparison with the control group of rats. The administration of glyproline compounds Thr-Lys-Pro-Arg-Pro-Gly-Pro, Pro-Gly-Pro and Pro-Gly-Pro-Leu contributed to a decrease in this indicator by 2.2 times ( $p \leq 0.01$ ), by 1, 7 ( $p \leq 0.01$ ) and by 1.5 times ( $p \geq 0.01$ ), respectively, in relation to the group of the stressed animals.

The level of caspase-8 in the group of the stressed animals with a submissive type of behavior increased by 2.4 times ( $p \leq 0.01$ ) in relation to the intact animals. The administration of glyproline neuropeptide compounds Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu contributed to a decrease in the level of the studied indicator by 1.9 ( $p \leq 0.01$ ), by 2.4 ( $p \leq 0.01$ ) and by 1.3 times ( $p \geq 0.01$ ), respectively, compared with the "social" stress group.

Table 2 presents the results reflecting the effect of neuropeptides of the glyproline structure on the level of TNF- $\alpha$  in the serum of white rats under conditions of "social" stress.

The formation of "social" stress in the animals with an aggressive type of behavior led to an increase in the level of the tumor necrosis factor by 45% ( $p \leq 0.01$ ) in comparison with the control group. Against the background of the administration of glyproline compounds Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro

and Pro-Gly-Pro-Leu, a decrease by 30% ( $p \leq 0.01$ ), by 25% ( $p \leq 0.01$ ) and by 22% ( $p \leq 0.05$ ) was observed, respectively, in relation to the group of stressed individuals.

In the group of rats with a submissive type of behavior, during the stress formation, the level of TNF- $\alpha$  increased by 52% ( $p \leq 0.01$ ) in comparison with the control. The compounds Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu caused a decrease in this indicator by 33% ( $p \leq 0.01$ ), by 22% ( $p \leq 0.05$ ) and by 23% ( $p \leq 0.05$ ) in relation to the "social" stress group.

Fig. 1 shows the results reflecting the effect of glyprolines on the NGF serum level of white rats under "social" stress.

In the group of animals with a "social" stress type of behavior and aggressive ones, a decrease in the NGF level by 40% ( $p \leq 0.01$ ) was observed in comparison with intact rats. The administration of glyprolines Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu increased the level of the studied factor by 40% ( $p \leq 0.01$ ); by 20% ( $p \leq 0.05$ ) and by 17% ( $p \leq 0.05$ ), respectively, compared with the "social" stress group.

The formation of "social" stress in the group of animals with a submissive type of behavior led to a decrease in the NGF level by more than 30% ( $p \leq 0.01$ ) in comparison with the control group. Against the background of the glyprolines administration (Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu), an increase in the level of the nerve growth factor by 56% ( $p \leq 0.01$ ), by 36% ( $p \leq 0.01$ ) and by 29% ( $p \leq 0.01$ ), respectively, compared with the "social" stress group, was notified.

Fig. 2 shows the results reflecting the effect of glyprolines on the level of the neurotrophic BDNF factor in the blood serum of white rats under the conditions of "social" stress.

In the group of stressed animals with an aggressive type of behavior, a decrease in the level of the brain neurotrophic factor by 40% ( $p \leq 0.01$ ) in comparison with the control group was notified. The Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu compounds increased the level of the brain neurotrophic factor relative to the stressed group of the animals by 45% ( $p \leq 0.01$ ); by 26% ( $p \leq 0.05$ ) and by 24% ( $p \leq 0.05$ ), respectively.

In the group of stressed rats with a submissive type of behavior, a 45% decrease in the BDNF level ( $p \leq 0.01$ ) was notified in comparison with control animals.

With the administration of glyproline compounds, the changes in the level of the studied neurotrophic factor were also notified in the form of a statistically significant increase ( $p \leq 0.01$ ): against the background of Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank) – by 52%, Pro-Gly-Pro – by 35% and Pro-Gly-Pro-Leu – by 32% in relation to the group of the animals exposed to "social" stress.

**Table 1 – The level of caspase-3 and caspase-8 in the blood serum of white rats under conditions of experimental “social” stress influenced by neuropeptides of the glyproline structure**

Groups of experimental animals	Caspase 3 (pg/ml)	Caspase 8 (pg/ml)
Control	17,41±1,22	2,33±0,91
Animals with aggressive behavior		
"Social" stress	30,62±2,13**	6,14±1,21**
"Social" stress + Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank)	19,22±2,01##	2,78±0,76##
"Social" stress + Pro-Gly-Pro	20,61±2,23##	3,65±0,56#
"Social" stress + Pro-Gly-Pro-Leu	23,76±2,14##	3,98±0,82
Animals with submissive behavior		
"Social" stress	27,83±2,21**	5,64±0,87**
"Social" stress + Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank)	18,67±1,21##	2,96±0,89#
"Social" stress + Pro-Gly-Pro	22,57±2,13	2,36±0,81##
"Social" stress + Pro-Gly-Pro-Leu	25,43±2,87	4,36±0,99

Note: \*\* –  $p \leq 0.01$  – relative to control; #, ## –  $p \leq 0.05$ ;  $p \leq 0.01$  – relative to the “social” stress group.

**Table 2 – The level of TNF- $\alpha$  in the blood serum of white rats under conditions of experimental “social” stress influenced by neuropeptides of the glyproline structure**

Experimental groups of animals	TNF- $\alpha$ (pg/ml)
Control	78.65±6.8
Animals with aggressive behavior	
"Social" stress	113.83±8.2**
"Social" stress + Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank)	79.32±6.8##
"Social" stress + Pro-Gly-Pro	85.60±8.1##
"Social" stress + Pro-Gly-Pro-Leu	88.77±7.4#
Animals with submissive behavior	
"Social" stress	119.35±7.8**
"Social" stress + Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank)	79.87±6.5##
"Social" stress + Pro-Gly-Pro	93.15±8.6#
"Social" stress + Pro-Gly-Pro-Leu	91.77±8.2#

Note: \*, \*\* –  $p \leq 0.05$ ;  $p \leq 0.01$  – relative to control; #, ## –  $p \leq 0.05$ ;  $p \leq 0.01$  – relative to the “social” stress group.

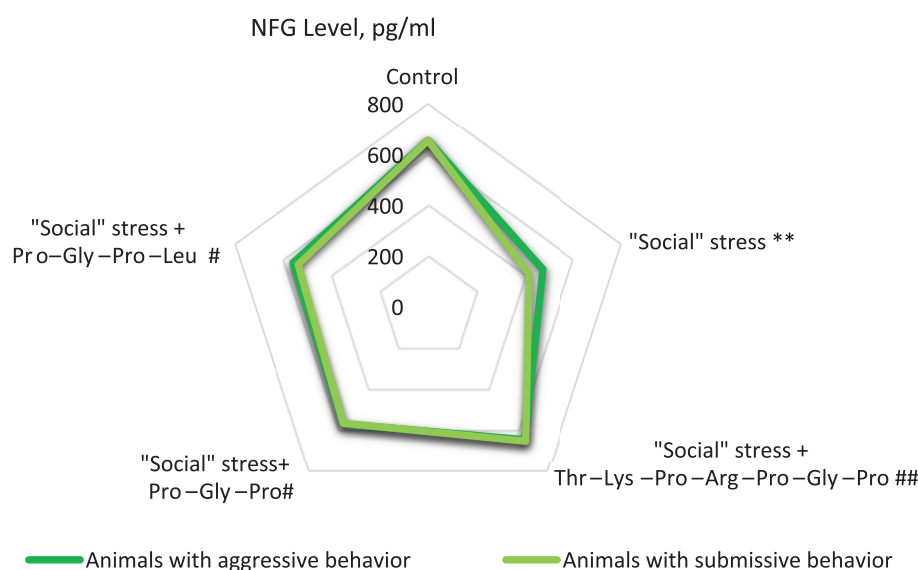
## DISCUSSION

In the course of this study it was established that “social” stress is accompanied by a decrease in the level of the brain neurotrophic factor and the nerve growth factor, which is associated with a change in neuroplasticity with a subsequent inhibition of neurogenesis. A number of experimental studies have shown that BDNF has pronounced neuroprotective properties, contributing to the inhibition of cell apoptosis, preventing, in turn, the neuronal death and stimulating the growth of cholinergic nerve fibers [42, 43]. It has been found out that under the conditions of “social” stress, alongside with a decrease in the levels of neurotrophic factors, there is an increase in the levels of caspase-3 and caspase-8, as well as the tumor necrosis factor in the blood serum of white rats. According to the literature data [44, 45],

such a variability of these indicators shows an increase in apoptotic processes.

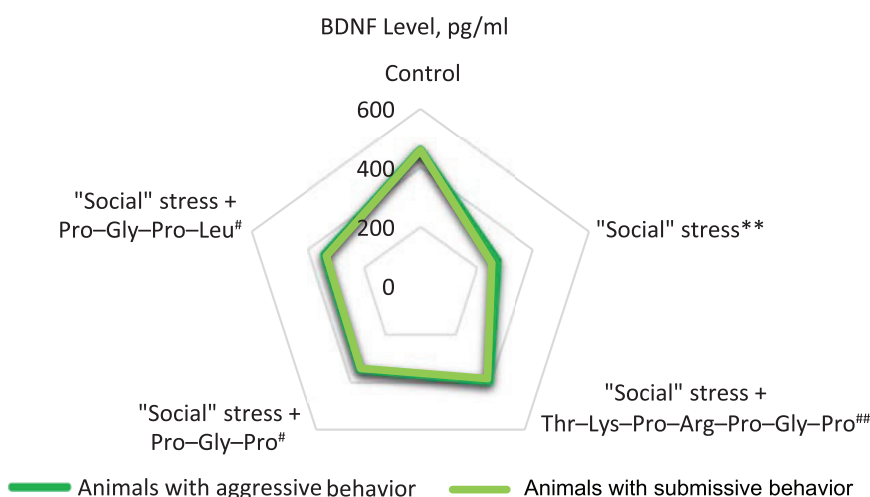
The essential role of neurotrophic factors in the induction or inhibition of apoptosis has been proven in other experimental studies. It has been established that NGF inhibits apoptosis in a number of neurodegenerative diseases [20]. In addition, it has been proven that the nerve growth factor and the brain neurotrophic factor implement their action both directly and through the genetic mechanisms of induction of apoptotic processes [5]. In contrast, a number of cytokines, in particular human interferons and a tumor necrosis factor, presumably have a stimulating effect on apoptosis [9]. That had also been confirmed in the authors' experiments before [36].

A decrease in the expression of neurotrophic fac-



**Figure 1 – The nerve growth factor level in the blood serum of white rats under conditions of experimental “social” stress influenced by neuropeptides of the glyproline structure**

Note: \*\* –  $p \leq 0.01$  – relative to control; #; ### –  $p \leq 0.05$ ;  $p \leq 0.01$  – relative to the “social” stress group.



**Figure 2 – The brain neurotrophic factor level in the blood serum of white rats under conditions of experimental “social” stress influenced by neuropeptides of the glyproline structure**

Note: \*\* –  $p \leq 0.01$  – relative to control; # –  $p \leq 0.05$  relative to the “social stress” group; ### –  $p \leq 0.01$  – relative to the “social stress” group.

tors as a result of stressful influences of various kinds of nature and the restoration of its level by a prolonged administration of corrective agents, led to the creation of a neurotrophic hypothesis for the development of stress-induced depression. According to this concept, a change in the level of neurotrophic factors is a key mechanism for the formation and development of approaches to the treatment of such disorders [17]. This fact is confirmed by the established decrease in the level of the nerve growth factor and the brain neurotrophic factor during the formation of a depressive state, and by an increase in the process of pharmacotherapy, as well as a positive correlation of the levels with the degree of the state improvement [7]. It has been proven that the effectiveness of antidepressant and stress-protective therapy is achieved due to the effect of drugs on the intensity of

neurogenesis and neuronal plasticity [24]. According to the literature data [25], the established corrective activity of glyproline neuropeptides in relation to the level of neurotrophic factors under a “social” stress, indicates the manifestation of Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu expressed anti-stress and neuroprotective effects.

Alongside with this, it has been established that the administration of glyproline neuropeptide compounds Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu against the background of “social” stress contributes to a decrease in the level of apoptotic indicators - caspase-3, caspase-8 and the tumor necrosis factor. This is mediated by the possible inhibition of the caspase-dependent cascade of reactions to the destruction of cellular structures by

hydrolysis of the nuclear lamina, cleavage of adhesive proteins and a destruction of the cytoskeleton. In the case of a caspase-dependent pathway, the signal for the onset of the programmed death of neurocytes is pathogenetic pathways formed upon the exposure to hypoxia, the agents of various kinds of nature (stressful, physical or chemical, etc.) [10, 11]. This pathway, alongside with caspases, is realized due to the binding of the tumor necrosis factor to receptors on the target cell membrane. Previously, the presence of the antioxidant action and the ability of neuropeptides to influence the level of pro- and anti-inflammatory cytokines have been proven [46]. It has been established that under the conditions of "social" stress, neuropeptide compounds induce a pronounced inhibition of free radical oxidation processes and reduce the concentration of proinflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$  [46, 47]. Based on the results obtained, it can be concluded that glyprolines have an anti-apoptotic action due to the effect on the level of caspases, the concentration of pro-inflammatory cytokines and the inhibition of lipid peroxidation processes.

The regulation of apoptotic and neurotrophic processes is complex; it involves various cytokines within

a large number of signaling cascades, which requires a further detailed study [48, 49].

### CONCLUSION

Thus, at present, focused attention is paid to assessing the role of apoptotic and neurotrophic factors in the implementation of the stress response. In this connection, an effector caspase-3 and an initiating caspase-8, a tumor necrosis factor, as well as neurotrophic factors (the nerve growth factor and the brain neurotrophic factor), are actively studied as targets for the action of stress-protective drugs of a neuropeptide structure in various pathological conditions, including those caused by a prolonged exposure to stress factors. The carried-out study has established the presence of an antiapoptotic activity in Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank), Pro-Gly-Pro and Pro-Gly-Pro-Leu due to the inhibition of the caspase-dependent cascade of apoptosis reactions. Alongside with this, a pronounced stress-protective effect has been determined due to the restoration of the level of brain neurotrophic factors. The obtained results actualize a further detailed study of the caspase-dependent and neurotrophic factors-mediated mechanism of the anti-stress effect of glyprolines.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### AUTHORS' CONTRIBUTION

Anna L. Yaseniyavskaya – collecting literature data, text writing, experiment setting up, the results obtained analysis, preparing a draft manuscript; Alexandra A. Tsibizova – collecting literature data, experiment setting up, the results evaluating, substantiating and statistically processing of the data obtained, preparing a draft manuscript; Liudmila A. Andreeva – synthesis of compounds, research planning, manuscript editing, results evaluating; Nikolai F. Myasoedov – synthesis of compounds, research planning, manuscript editing, results evaluating; final approval for manuscript publication; Ol'ga A. Bashkina – research planning, manuscript editing, results evaluating, final approval for the manuscript publication; Marina A. Samotrueva – concept development and study design, study planning, experiment setting up, critical intellectual content reviewing, and final approval for manuscript publication.

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